V.

T H E S I S

for Degree of M.D.

on

THE DECOMPOSITION OF FISH AND ITS DETECTION FROM A PUBLIC HEALTH POINT OF VIEW.

by

A. G. ANDERSON,
M.A., M.B., Ch.B., B.Sc., D.P.H.,

Resident Physician to City Hospital, and
Assistant to M.O.H., City of Aberdeen.

I N D E X.

During the past two years, in my capacity as Assistant to the Medical Officer of Health, I have had frequently to deal with questions regarding Trawling, Fish-curing and the examination of Fish. Not infrequently have I been unfavourably impressed not only with the lack of knowledge exhibited of the most elementary scientific principles underlying the various processes in operation in the different Departments of this great industry, but also with the absence of the spirit of scientific enquiry which is so essential in any progressive business. Hence, to stimulate the spirit of enquiry amongst such people, I commenced to write these articles, with the hope that, not only Fishermen, Fish Curers and Meat Inspectors might gain a more intelligent acquaintance with their business, but that thereby the great fish-consuming public might derive considerable benefit.

This subject, although novel in some respects, is concerned, not only with one of our chief sources of food supply, but with a great Industry, which, like so many other industries in this country, is too frequently left to languish, for the want of scientific application.

The importance of Fish as a food stuff has, in recent years, been gradually gaining favour; and in order to supply this increased demand for edible and fresh fish, there has simultaneously sprung up the great Trawling Industry, which has largely displaced the older line-fishing.

Now fresh fish, from its essential nature, is a food stuff which readily undergoes decomposition and putrefaction. That these processes are associated with, and chiefly caused by micro-organisms, which are universally present, are facts now well understood and capable of easy demonstration, the three dominant factors which facilitate or inhibit their action being (1) Supply of Nourishment; (2) Temperature; (3) Degree of Moisture. Further, the Structure and Chemical Composition of the muscular tissue in fish is such as facilitates the easy invasion of Bacteria, and the early onset of decomposition. Regarding structure, we find that in mammals the striped or red variety of muscle predominates, whereas in fish it is the pale muscle which exhibits a lower grade of differentiation than the former. In pale muscle the striations are less regular, and the fibres much more readily break up into smaller filaments, and these again into discs. The fibres are not bound into strong bundles to form distinct muscles as in the red muscle in mammalia, they are simply separated from each other by delicate connective tissue septa; while pale muscle is also slightly less vascular.

When we pass to the chemical composition, we find, that, with the exception of some fish, such as Salmon, Eel and Mackerel, in which the proportion of fat and water does not differ materially from that in red muscle, the flesh of pale muscle of the ordinary round and flat fishes is comparatively free of fat, but contains a much larger proportion of water than the red. The amount of proteids, on the other hand, in pale muscle does not appreciably differ from that in red muscle.

Hence, when we compare the pale muscle of fishes with the red muscle of mammals, and find that in the former the amount of fat and water are the most variable quantities; while, at the same time, the tissues are more open in texture, with delicate connective tissue septa and slightly less vascular, it is probable that these properties form some intimate relation with the greater susceptibility of such tissue to the attack of putrefactive micro-organisms and decomposition, than that shown by mammalian muscle.

The question of the presence or absence of Bacteria in the blood and tissues of healthy living fish has recently received some consideration. I think it is generally recognised that micro-organisms, as also many of the well known parasites, do find a habitat in the tissues of these lower animals, and that to a far greater extent than in the tissues of higher animals. During microscopic examinations of blood, I have occasionally observed rod-like and coccus-like bodies, which, although suggestive of Bacteria, I have not as yet been able to isolate

by culture. I have frequently examined , both microscopically and by cultures, the fresh peritoneal fluid of fish, when removed immediately after death with aseptic precautions, and when due care is taken, I have not as yet found any micro-organisms in this fluid. Neither in the fresh, healthy, living, muscular tissue have I ever found any micro-organisms, when examined in the same way with due precautions. Consequently, I am persuaded that, excluding the gut, micro-organisms do not exist to any appreciable extent in the tissues, nor body fluids of fish under normal conditions, although it may be possible that, under any abnormal conditions, the tissues may be unable to destroy some of the Bacteria which reach them during life, or, since the tissues of these animals have great powers of adaptation, they may become tolerant to some. But after death, the tissues of fish offer comparatively little resistance to the invasion of putrefactive organisms. For soon these will be found multiplying in great numbers in the gut and in all the body fluids and gradually penetrating in amongst the surrounding tissues.

Among the products of Bacterial activity are some substances of an alkaloidal nature which are very poisonous and have recently been isolated from decomposing fish. It is found that one class of these alkaloids, to which the name mytilotoxin has been given, acts chiefly on the nervous system, paralysing motor nerves like Curara, while another class of these acts chiefly on the digestive organs, causing acute Gastritis and Interitis. Hence, when one has regard not only to the

increasing quantity of Fish used as food, but also to the susceptibility of fish to decomposition, and to the advent of the Trawling Industry, where fresh fish may remain in an iced condition for one to two weeks or longer, the detection of the earlier stages of decomposition in fish is a matter of the highest importance.

This appeals most to Public Health Authorities, whose concern is the public health, and especially to the Inspector of Meat. To him the examination of fish for the purpose of determining their condition from one of freshness to putridity, must often impress the sober truth of the adage - "Appearances are deceptive." When Fish are seen newly caught, or when putrefaction is pronounced, the appearances in each case are sufficiently characteristic and definite. But between these extremes, and especially in the incipient stages of decomposition the question is often one of great difficulty and doubt. Here the usual tests employed are very arbitrary, and it is impossible to fix any standard towards which all would agree; whereas when a Fish has reached the stage of putridity, any ground for contention disappears.

It was in consideration of the above mentioned difficulties and at the suggestion of Professor Hay, that, during 1907, from beginning of August to the end of December, the following observations and experiments were made at the Bay of Nigg, Fishery Board Station and in the Laboratories of Marischal College, with a view of determining the value of the more common tests applied

in examining Fish, and, if possible, to find any others more readily applicable and reliable. The Fish experimented with were Haddocks, Whitings, Sole, Plaice, Dabs, Cod and Herrings.

The method adopted was to procure both line fish and trawl fish by prearrangement from reliable Fishermen, who carefully noted time of capture and location. The Fish on landing were laid out on trays. Some were washed daily with sea water and kept covered with a damp cloth, in such conditions as might prevail in a Fishmonger's shop, while others were subjected to different methods of treatment. The Temperature was carefully noted from day to day, as the fish were always kept at the ordinary atmospheric temperature.

I will now discuss seriatim the different criteria as usually applied in the examination of Fish.

I. General Appearance of Fish.

II. Handling the Fish.

III. Appearance of Surface and Scales.

IV. Appearance of Eyes.

V. Appearance of Gills.

VI. Smell.

VII. Discoloration on Ventral aspect of Backbone.

VIII. Rigor Mortis.

IX. Manner in which flesh strips away from the Backbone, or bone away from the flesh.

X. Part played by Gut and appearance of Abdominal Walls.

GENERAL APPEARANCE.

A little experience soon teaches one that it is not only often extremely difficult, but often impossible, to determine by appearance alone whether any given Fish is or is not fresh and fit for human food, and in this respect there are many possibilities of error. This is often the case with Trawled Fish which have been dragged for some time in the trawl net over a muddy or rough sea floor, or may have been imperfectly iced. The body region of such Fish usually presents a very battered and limp appearance, while the head shows more or less extravasation of blood. They may look unsaleable and quite unfit for human food, but if more closely examined, may be found quite fresh. On the other hand, not infrequently in the case of flat fish, on account of their tougher skin and firmer texture, they often appear quite firm and fresh, when on closer examination, the tissues are saturated with sour-smelling ferments, and in the earlier stages of decomposition.

HANDLING A FISH.

One carefully notes the presence or absence of rigidity - especially towards the tail region, where it generally persists longest. If it is present, it stamps the fish as perfectly fresh, and they will be found to be firm and elastic to touch and to slight pressure between finger and thumb. If it is absent, then the fish are not quite fresh. Instead of being

firm and elastic, they become soft and inelastic, and very soon pit readily and deeply on moderate pressure. Their fitness or unfitness for human food have to be decided by other considerations. There are also certain very definite chemical changes, which take place as muscle passes from the fresh to the putrid condition, and which can be readily detected by litmus paper. But this subject will be discussed in detail subsequently under the heading of Rigor Mortis.

APPEARANCE OF SURFACE AND SCALES.

One readily observes the imbricated arrangement and disposition of the Scales, with their silvery, iridescent and golden sheen below the lateral line, and paler olive colour towards the dorsum, when a fish is seen newly caught and perfectly fresh. This appearance is, however, only of hourly duration and the lustre disappears long before decomposition ensues. But more important is the general firmness or looseness of the scales. In the fresh state the scales have a certain degree of firmness, and hence when one finds that they rub off readily, it certainly indicates that the fish are not quite fresh. If, on the other hand, the surface presents a patchy appearance, it often indicates that the fish have been trawled or roughly handled. This appearance, combined with a good deal of blood extravasation about the head region, are very characteristic of trawled fish.

APPEARANCE OF THE EYES.

This readily appeals to most people in examining Fish, but it has a very limited value. In the newly caught and fresh fish, the full and prominent eye, with jet black pupil in most fish, and transparent cornea, is a very prominent feature. In whatever condition the fish is kept, these appearances are very brief. For in 24 hours, in most cases, one can detect commencing opalescence in the corneae, with a lack-lustre appearance of the pupil, and usually by 48 hours, slight hollowing of the eyeball is seen. These changes gradually become more intensified, and by the 3rd or 4th day the eyes are grey and shrunken.

APPEARANCE OF GILLS.

This is a time honoured criterion, and although a good one, yet it is not an absolutely safe guide regarding the condition of a fish. In fresh fish, the colour of the Gills is described as bright red. This may be a good generic term, but in Haddocks and Whitings, although the ground colour is red, it is not a deep red, there is present a quite characteristic pale reddish tint, whereas in the Herring it is a darker red or brownish red tint. Thus different varieties exhibit different tints with red as a ground colour. (See Diagrams on last page). Now in every case in about 24 to 36 hours, the gills in all varieties of fish begin to lose their reddish tint and gradually to become grey and slimy. This always occurs by the 3rd or

4th day.

There are, however, certain points which have to be kept in view. In examining quantities of fresh fish, one meets frequently some which have paler gills than their neighbours, and yet are perfectly fresh, and it is remarkable how in many cases the gills retain with little diminution - especially if washed daily with fresh water or salt water - their characteristic tints, even when the fish have become putrid. Also the gills of trawled fish at time of capture, more especially if they have been dragged in the trawl net for some time, are usually of a paler colour than line fish at time of capture.

SMELL.

So long as fish are fresh, they retain their characteristic but not disagreeable odour. But when fish begin to decompose through Bacterial activity, new substances are formed, which are often characterised by disagreeable penetrating odours, and often the escape of these volatile bye-products is the first warning that decomposition has set in.

There are two stages in the history of a fish on the high way to decomposition concerning which all will agree. First, when the odour is perfectly fresh and natural, and secondly when the odour is putrid; in the former condition, the fish are fit for human food, in the second condition, they should be unhesitatingly condemned. But there is an intervening period bridging between these extremes; and here it is the daily

experience of those engaged in the examination of fish, how difficult often to interpret these odours correctly as regards the indications which they may give concerning the condition of a fish, and its consequent fitness or unfitness for human food. It is in this intermediate stage where there is room for contention and disagreement.

Regarding this subject, I have made the following observations:-

(1) That in every case unwashed fish give off an offensive odour sooner than washed fish. Hence, in smelling an unwashed fish, an offensive odour might be derived from decomposing slime lying on the surface, although the fish itself might be quite fresh.

(2) If fish are washed daily with sea water, or even tap water, the development of an offensive odour is considerably retarded.

(3) Ungutted fish soon develop a very disagreeable odour from the decomposition which rapidly ensues, especially in the gut, and to a less extent in the liver.

(4) If any fish gutted or ungutted in the incipient stage of decomposition, and giving off a slightly tainted odour, are thoroughly washed in sea water, it is remarkable how they are freshened. The tainted odour is expelled and for a time they may again smell quite fresh.

(5) Trawled fish are comparatively free from slime when taken on board, because they have been dragged along for some

distance through the water at a considerable speed and the most of the slime washed off. Still such fish when removed from ice soon begin to give off a tainted odour, because decomposition generally sets in earlier than in line fish.

(6) To test a fish fairly, it is always necessary to assure oneself that the smell is derived from the flesh, the skin or the slime, or all combined.

However, regarding the sense of smell as a criterion for purposes of meat inspection, one has always to keep in view, that the sense of smell is differently developed in different individuals, and that it is impossible to set up any exact standard of smell. The different terms used, such as Fresh, Tainted, Putrid, etc., can only have a relative value, although a general standard is always understood. Still I think it is possible to place the more common edible fishes into two groups, the one containing the Haddock, Whiting, Turbot, Halibut, Plaice and Dabs, etc., the other containing such as the Salmon, Eel, Herring, etc. Now, both these groups possess a very characteristic odour when removed from sea water. The former may be described as Fresh, Fishy and sea-weedy; the latter as Fresh, Fishy but oily.

The time taken before the fresh, fishy odour becomes tainted, stale and finally putrid, depends, as already stated, on the suitability of the media, the degree of moisture and temperature for the Bacteria of putrefaction. The process and stage proceed along perfectly definite lines and are the same

for all fish.

In the case of washed, ungutted Haddocks and Whitings experimented with during last July and August, I invariably found the fresh, fishy odour beginning to be tainted after 48 hours, distinctly tainted and stale after 60 hours and becoming putrid by 72 hours. With washed, gutted Haddocks and Whitings the time is longer before the tainted odour begins to be appreciable, but on an average about 60 to 72 hours, and by 84 hours it is distinctly putrid. As regards Herrings, the time of appearance of tainted odour appears to be more variable; in some cases after 33 hours, in others not until about 50 hours, but, on the whole, it is earlier than in the case of most white fish.

REDDISH DISCOLORATION ON VENTRAL ASPECT OF BACKBONE.

In all the fish examined, there appeared with striking regularity a reddish-brown discoloration on the ventral aspect of the backbone, usually between the second and third day in the case of line fish, and on the whole earlier in the case of trawled fish. It is best seen in the region extending from the kidney to the tail. The kidney itself is a diffuse reddish organ, lying on ventral aspect of anterior region of the backbone. It is very friable and after death readily disintegrates to form a reddish debris in this region, but is not to be confused with the reddish discoloration round the vertebral column, which has a different origin and a different significance.

The earliest appearance of this thin red line in line-caught fish was about 48 hours after capture. It gradually increases in size from $\frac{1}{8}$ to $\frac{1}{4}$ inch in diameter during the following 12 hours and is usually well seen after 60 to 72 hours. Occasionally it was observed in trawled fish before 48 hours after landing, and on the whole, it appears earlier and develops quicker than in line fish.

The regularity of this appearance suggested a daily microscopic examination of the blood in order to ascertain if there were any changes taking place in it, which in any way might be correlated with, and which might be considered explanatory of the former reddish discoloration.

Taken from the fresh fish, the red corpuscles are seen to be oval and round in outline, with a reddish-yellow colour, showing prominent nuclei and nucleoli. The white corpuscles show no special feature. Occasionally a body will be seen which appears to contain uncysted sporozoa, and occasionally a slender, sometimes a thick rod-shaped bacillus-like body or a few coccus-like bodies may be seen lying in the serum. On the second day the red cells will generally show a breaking up of their contents. The cell wall in many cases will show dimpling and creases, while in others the cell contents will shrink away from the cell wall leaving clear spaces, and a few Bacilli may be seen in each field. On the third day, that is after 60 hours, the blood now presents the appearance of an

amber coloured fluid. The corpuscles have nearly all broken down with escape of their contents. In each field there will now be seen many micro-organisms, some of which are motile and a few fragmentary corpuscles. The blood for examination was always taken from the cardinal vein in the caudal region, but by the third day the fluid in this vessel was very scanty.

Now the discoloration of the tissues which produces the appearance of a reddish-brown ventral line, will be observed to commence circumjacent to the caudal vein, and in that part of the vertebral column which forms the roof of the haemal arch. It then proceeds outwards until a column of tissue from $\frac{1}{4}$ to $\frac{1}{2}$ inch diameter is discoloured and usually the greater part of the vertebral column. If the muscular tissue round the vein be examined from day to day, the commencement and progress of the staining of the muscle fibres is readily seen, and by the third day 60-72 hours, micro-organisms are readily detected amongst the tissues, although none will be found in the tissues immediately after death, when examined with proper precautions.

These examinations were made by both wet, dried and stained microscopic preparations, and by ordinary culture media. The extended Bacteriological examination as regards the nature and properties of these micro-organisms will form the subject of another paper.

From these investigations it appears that the micro-organisms present in the blood before death, as also those that gain entrance after death, multiply rapidly in the blood which

forms a suitable nutrient medium, while at the same time the red blood cells disintegrate, and their colouring matter or Haemoglobin is set free. Both micro-organisms and colouring matter soon make their way through the wall of the blood vessel. The former can be detected amongst the tissues, while the latter causes the reddish brown staining of the tissues and forms the red line in the ventral aspect of the backbone. It will be noticed that this staining does not increase beyond certain limits as putrefaction proceeds, the reason being that as the blood is limited in quantity, so also must its staining powers. These changes occurred in all the fish examined, such as Haddocks, Whitings, Cod, Herring, Plaice, etc., and it occurs in gutted and ungutted fish alike.

The chief value to be derived from the observation of the presence, degree of development or absence of this red line, is, that it indicates fairly accurately the length of time since the fish were captured or landed. Recently in some fishing districts it has been attempted to remove this large blood vessel along with the gut at time of capture. In the fish trade, haddocks with well marked red discoloration will not readily sell as fresh fish, and are usually cured. When cured, the red discoloration is still present, and such a fish will be slightly sour to taste and smell, and its keeping properties are impaired.

RIGOR MORTIS.

The study of Rigor Mortis in fish is a subject of no less

importance to those engaged in the inspection of fish in the interests of Public Health, than to Trawl Fishermen and Fishcurers, who are so often concerned in the preservation of fish as long as possible in the fresh state.

Physiology teaches, and it is a matter of simple experiment, that muscular tissue retains its property of irritability and will therefore respond to stimuli for some time after the death or destruction of the brain and the cessation of all voluntary movements. The stimuli may be mechanical, as pinching, cutting, etc., the electrical, such as may be produced by a galvanic cell. This is well seen in the lower subkingdoms such as pisces and amphibia. Since the muscles in these cold blooded animals are not so closely under, nor so dependent on cerebral control during life, and since in them metabolic changes are not so active, they on this account exhibit a greater vitality after death, and retain their property of irritability longer than in higher animals. Then as rigor mortis can only supervene after the complete cessation of irritability, it is consequently later in appearing and longer in disappearing in these animals than in mammals or birds. As the due appreciation of these facts would be invaluable to Trawl-fishermen, Fishcurers and Meat Inspectors, and since such knowledge could be utilised on the one hand for the better preservation of fish, and on the other for the inspection of fish, the following simple experiments may be readily carried out, and by doing so, an intelligent and practical acquaintance with this

subject may thus be readily obtained.

When a newly caught fish is taken out of the water, as in rod or line-fishing, it leaps and wriggles about, often with fins erect, and attempts to get back to its natural habitat. These movements gradually diminish, and usually in 15 to 30 minutes have ceased, and in 5 minutes more, there is usually no response on handling. The fish is now practically dead, but the muscles still retain their power of irritability for a varying length of time, which may extend from 10-15 hours according to circumstances, and will respond in the form of contraction to electrical and mechanical stimuli, which may be produced, on the one hand, by a very simple electrical apparatus and on the other, by simply tapping, pinching, cutting, etc. This property of irritability will be found to disappear first in the muscles of the head region, then in those of the trunk, and lastly in those of the tail region. Then just in the same order from before backwards, the gradual disappearance of irritability is succeeded by rigidity of the muscles or Rigor Mortis. It is first seen in the muscles of the lower jaw and gill covers, when the mouth and gill covers are firmly closed. The stiffening then travels backwards until the whole fish is rigid, and when complete, the mouth is often gaping widely open. After an interval of time, varying from hours to days, the rigor begins to disappear, and precisely in the same order as it appeared: first, the muscles of the jaws and gill covers, then the trunk, and lastly those of the tail region, until the whole

fish becomes quite soft and limp, just as it was when removed from the water.

Such, in general outline, is the sequence of events, but there are, however, many important factors which exercise a determining influence as regards time of onset, length of duration and disappearance of rigor in fishes. The most important of these I will state briefly. They are from observations made on a large number of fish.

The cause of Rigor Mortis - the coagulation of the soluble myosinogens of the muscle plasma - is not for the present under consideration.

It will be found that Rigor is later in appearing and lasts longer in the following conditions:-

A. Fish in season.

B. Fish in a healthy and vigorous condition.

C. Fish which are at once killed on capture.

D. Fish which are not only killed but are pithed at the same time - that is have the brain and spinal cord destroyed.

E. Fish gutted immediately on capture.

F. Fish handled as little as possible after capture.

G. Fish kept at low temperatures, as when iced or kept in cold storage.

On the other hand, Rigor appears earlier and disappears sooner in the following conditions:-

A'. Fish not in season.

B'. Fish in an exhausted condition.

C'. Fish not killed at time of capture.

D'. Fish neither killed nor pithed at time of capture.

E'. Fish ungutted.

F'. Fish roughly handled.

G'. Fish uniced and not kept at low temperatures.

It is also to be observed that Rigor tends to persist longer in those varieties of fish, such as salmon, whose muscular tissue is firmer in texture and contains a smaller percentage of water than in most varieties of white fish, as whitings and haddocks, where the tissues are not so firm and contain more water.

From these observations, we must conclude that the degree and duration of Rigor in fish depends chiefly on the condition of the muscular tissues at time of death. The more the conditions at time of death approximate A, B, C, D, E, F, G, the later will rigor - sometimes 10-30 hours - set in, and it may persist 1-3 days; whereas the more exhausted the fish is, when conditions A', B', C', D', E', F', G' obtain, the sooner it appears and disappears, and is sometimes even difficult to detect.

The cause of the disappearance of Rigor in muscle is a question regarding which all are not agreed. True it is, that, in muscle in condition of Rigor, the conditions for pepsin-

digestion are present. The muscle is acid and pepsin ferment, although in very small quantity, is also present. Still it appears that although pepsin-digestion may play some part in the initial stage, it is a small one, and that the chief and final cause is due to Bacterial invasion. It is very rare to find any micro-organisms in muscle during rigor, but as rigor passes off, they increase rapidly.

As already stated, to preserve fish as long as possible in rigor, conditions A, B, C, D, E, F, G, have to be observed, and in practice the most important is G - the maintenance of Low Temperature.

Since it is possible to inhibit the action of most Bacteria of putrefaction by maintaining a low temperature from 0°C. to -3°C., while at the same time maintaining the fish in a condition of Rigor, it thus becomes possible to preserve fish in a comparatively fresh condition for a considerable time with very little deterioration in their tissues. At temperature below -3°C. the fish suffer considerably. When such fish are thawed, they are usually found to be very soft and limp, and pit deeply on pressure. The lower the temperature, the deeper and more extensive the freezing of the water in the tissues, which must cause at the same time a proportionately greater amount of mechanical disintegration. Such fish are more difficult to cure, when cooked, have lost much of their natural flavour, and very readily undergo decomposition. By maintaining the temperature about -4°C to -5°C, fish appear to remain in a condition of Rigor indefinitely. If the temperature is kept at

-5°C, or lower, fish do not appear to pass into rigor, but may be observed to do so on raising the temperature above -4°C.

It has so happened that on some occasions the fish I was experimenting with were kept in a mixture of sawdust and ice. This combination impressed me favourably, and it appears to be more effective in maintaining rigor and inhibiting the onset of decomposition than ice alone. The best mixture appears to be small lumps of ice with intervening spaces filled up with sawdust. Although I have not been able as yet to experiment very far in this direction, yet I am convinced that this matter deserves consideration and a fair trial by those engaged in the trawling industry.

Further, there is the very important question - what is the best time to ice fish? -

A. When Rigor is completed?

B. Before Rigor has set in?

C. After Rigor has disappeared?

In the trawling industry, the process of icing is necessarily and so extensively practised, that the general conditions of freshness, curing and edible properties of the fish may with truth be said to depend on its proper performance. Consequently, this is a subject which deserves more consideration than it has hitherto received. But as this part of the investigation is not yet complete, and as many enquiries are in hand as to how far these conditions are observed and practised in Trawling, I will only add a few conclusions which I have already arrived at.

During the past few months I have carried out a series of experiments, chiefly with Haddocks and Whitings. Some were gutted, some left ungutted, some killed, gutted, pithed, etc., so as to vary the conditions. Then some of each variety were placed in ice under condition A, when Rigor was completed. Some were iced under condition B, immediately after death, before Rigor had set in; some under condition C, at different periods from 1 to 10 hours, after Rigor had passed off. Then some of each A, B, C, were removed at intervening periods of 5, 10 and 15 days afterwards, examined and treated so as to ascertain their keeping, curing and edible qualities. These were found to be best preserved under condition A; and in the second degree under condition B, while in the lowest degree under condition C. Also, under condition C, the longer after Rigor had passed off, when the fish were iced, in proportion the more readily did they undergo decomposition, when removed from the ice.

Detection of Rigor Mortis.

When vigor is strongly and fully developed, its presence is easily detected. The fish is quite rigid or nearly so according to the degree of rigor, which again depends on conditions already discussed. When one balances such a fish on the finger, it may remain quite rigid, but when rigor is beginning to pass off, it may begin to droop at head or tail or both. Here it is often difficult to be certain about the condition and especially when rigor has not been well developed.

But now one will observe that the fish from being firm and elastic to touch becomes softer and inelastic, and pits readily on pressure. The chemical changes are always fairly definite during these transitions. Preceding Rigor, while the muscles are irritable and respond to stimuli, their reaction is neutral or faintly alkaline. During Rigor, they are strongly acid. As Rigor passes off, they become neutral. Then as decomposition ensues, they become strongly alkaline, when tested with litmus paper.

This part of the subject has been discussed in more detail than originally intended in this paper, because so often do we find, on the one hand, that those who have to do with the preservation of fish in the fresh state, have not given the subject due consideration, nor recognised its great commercial value; and yet it is the initial and most important stage in the process of fish preservation: while, on the other hand, this subject is often equally ill-understood by those whose duties are to examine fish in the interests of Public Health, and they likewise fail to take advantage of an invaluable criterion, which, when present, stamps fish as absolutely fresh, or, if absent, points the way to ensuing putrefaction and decomposition

Trawl Fish compared with Line Fish.

Since the advent of the great trawling industry, the question has often been discussed as regards the general condition, curing-properties and keeping-properties of trawl fish

as against line-caught fish. But, at the present time, opinion is very much divided.

Professor MacIntosh in Appendix to Report of Trawling Commission 1885, states that the general condition of Trawl fish is excellent and that they become rigid like line-caught fish, whereas, on the other hand, it is the experience of many Fish Curers, that it is often difficult to cure such fish, especially as Findon Haddocks.

Now, I have examined many lots of fish taken from the trawl net after being a certain number of hours at work, and treated under different conditions. Some were killed when taken on board, some not killed, some killed and pithed, some gutted and some ungutted. When such fish are compared with line fish under similar conditions, it will be found generally that rigor sets in earlier and disappears earlier in the trawl fish.

The reason for this is evident, when one considers the conditions under which fish are trawled, and compare these with the above table. The longer the fish remain in the trawl net, the more do they become crowded together, while the motion of the net through the water maintains a certain degree of compression. Respiration is consequently impeded while the fish are struggling to respire and to get freedom. The result is that the tissues are not sufficiently oxygenated, waste products accumulate, and the fish become more and more exhausted the longer they remain in the trawl net. Fishcurers frequently observe this condition in Herrings which have been caught under

similar conditions and speak of them as "drowned Herrings," since many of them are found dead when taken on board. These are always difficult to cure. It will be frequently found, however, that in examining a catch of trawl fish, many of them pass into a condition of Rigor and behave similarly under treatment as line fish. These have only been a very short time in the trawl net, and some may have been captured when the net was being hauled on board.

From these investigations, we must conclude that trawl fish, since Rigor sets in earlier and disappears earlier, and since it is immediately followed by decomposition, are neither equal to line fish in general conditions, nor in curing-properties, nor in keeping-properties, except when the fish have only been a short time in the trawl net.

The above statement compares trawl with line-fishing only so far as they are comparable, and takes no account of the process of icing fish, which is almost exclusively confined to trawling.

MANNER IN WHICH FLESH STRIPS AWAY FROM BACKBONE, OR BONE AWAY FROM THE FLESH.

When fresh fish are examined from day to day, it will be found that during the first day, it requires considerable pressure by finger and thumb to separate flesh from bone, or to strip bone from flesh; and in doing so many tags of flesh are left adhering to bone. By the second day, although rigor

mortis will generally have disappeared and softening commenced due to early stage of decomposition, yet there may not be much difference to first day. But from many observations made, it was generally found that, by the third day, the flesh is now much more friable and softer, and separates from the bone with moderate pressure. By the fourth day, the flesh will generally be found to be soft and pulpy and to strip off readily and cleanly, leaving very few tags adhering to bone. On the fifth day the flesh and bone separate from each other readily and cleanly.

When gutted and ungutted fish are compared together in this respect, it will be found that the difference in time when the flesh in both strips off alike is not so great as one might expect, although it is certainly longer on the whole in the case of the former than the latter. This is a very valuable test, but for its due appreciation some care and practice is required.

It would be as difficult as useless, however, to attempt to set any time limit, when a fish should be condemned by this test. The times stated above are an average, but will be found fairly accurate in practice. One must judge by the degree of pressure required, and the degree of cleanness of separation. When the flesh comes away readily and cleanly with little pressure, one usually finds other confirmatory signs and has no hesitation in at once condemning such fish.

PART PLAYED BY GUT AND APPEARANCE OF ABDOMINAL WALLS.

The part played by the gut is one of the chief factors in

initiating decomposition in fish and possesses considerable interest. If ordinary white fish, such as Haddock or Whiting, line-caught and ungutted, be laid out and kept moist for experimental purposes and examined from hour to hour, it will be found that the wall of the gut in almost every case is the first part to undergo post mortem changes and solution. This will sometimes be observed to take place before rigor mortis has set in, and very frequently before rigor has passed off. It is a question round which there has been much discussion, whether this solution of the wall of the gut is due to the digestive action of intestinal ferments or to putrefactive processes. But when one has due regard to the rapidity in many cases with which solution of gut wall takes place, it appears to be at least initiated by post mortem digestion, although this process may be accompanied by and is certainly soon superseded by the action of the Bacteria of putrefaction which abound in the gut.

There are, however, certain factors which appear to hasten or retard this process. It will always be found to take place sooner in fish which have been feeding immediately before capture. Amongst the fish under examination, one will frequently find, especially in dealing with Herring or Cod, some whose stomachs were evidently packed with crustaceans or small fish at time of capture, and in these cases digestion and solution of wall of gut may take place in a few hours; whereas if the gut is comparatively empty, the digestion may be considerably delayed. This certainly takes place very rapidly in

Herring in the above condition, and I have frequently observed it in Herring in the spent condition. It has frequently been observed by fishermen that Herring with stomachs packed full at time of capture, very rapidly undergo putrefactive changes, and often have been useless for curing purposes before being landed.

It occurred to me that possibly the kind of food might exercise some influence in determining the earlier or later onset of decomposition in fish. Accordingly I examined the stomach contents of a considerable number of fish. But it appears to me that fish, although they may have and do show some predilections for certain kinds of food, are on the whole indiscriminate feeders, and that the kind of food partaken of is determined chiefly by the habits and disposition of the individual fish, and the condition of hunger at time of feeding. Haddocks are not so agile swimmers nor so sharp sighted as Whitings. Consequently, they are not so good hunters as the latter, and hence one generally finds the principal food in the stomach of the Haddock is crustaceans. Whitings, on the other hand, are rapid and agile swimmers with keener eyesight. They hunt their food, and one finds that it consists chiefly of young Herrings, Sand Eels and small flat fish. Cod are voracious eaters and appear to feed on Crustacea and smaller fish. But on the whole, more of the former were found in their stomachs than the latter, and it is possible that they prefer the former to the latter, when it is readily accessible. As regards Saithe, I found that in the adult fish the stomach contents consisted of

mixtures of Herrings and many varieties of smaller fish. But the younger the saithe, they appear to prefer crustacea.

Herrings appear to feed largely on Crustacea and Sand Eels. I also examined the stomach contents of a few other varieties of fish, but it appeared evident that no very definite conclusions could be formed from this line of enquiry, since fish do not adhere to any one class of food, and that what they eat depends chiefly on the exigencies of circumstances as stated above. However, in order to control the conditions of feeding and make this enquiry more precise, I obtained a small cargo of Haddocks freshly caught, brought ashore in sea water and transferred at once to tanks, where they were kept in conditions approaching to their natural habitat. After a few weeks, when acclimatised and feeding readily, separate lots were fed for a few days on such foods as Bread Crumbs, pieces of Fish and Crustaceans. Then, on a certain day, so many were taken from each tank at different times after feeding and killed, some at 15 minutes, 1 hour, 2 hours, etc. These were laid out in plates and kept moist and observations made from hour to hour.

From a large number of observations, I came to the conclusion that the kind of food in the stomach exercises some, but not an important influence on the time of onset and rate of decomposition. Although as regards the fish fed on carbohydrates, digestion of wall of gut, when it did take place, was slower than in those fed on proteid foods, also on the average decomposition was slower in appearing. Still it has to be

kept in view that very few edible fish in their natural habitat feed on carbohydrates, so that this observation does not possess much of practical value.

Far more important, however, as regards the onset of decomposition is the time after feeding when the fish are killed. Invariably in those killed from 15 to 45 minutes after feeding, post mortem digestion appeared to be more active. Consequently in a greater number of these, digestion and solution of gut wall took place sooner than in those killed at a later period of from 1 to 2 hours after feeding. This observation is in accordance with Pawlow's experimental work on gastric secretion and digestion, - that increase in quantity of food and especially proteid food causes a more active secretion of gastric juice, and that the secretion is more abundant in the earlier stage of digestion, or soon after the ingestion of food.

This part of the enquiry, however, I think, must be studied in a wider and more natural field than within the confines of the Laboratory. Here the environment is so different to that which obtains in nature, and to which fish do not become readily acclimitised. I have often discussed this question with intelligent and observant Fisher people, who assure me that the keeping, curing and edible qualities of fish vary greatly with the nature of the ground on which they have been living and feeding. So much so is this the case that they associate soft ground with soft fish and hard ground with hard fish, and map out large areas of fishing ground accordingly. They are of

opinion that the food stuff chiefly obtained on the soft ground consists chiefly of worms and small fish, while that obtained on the hard ground consists largely of Crustacea, and this to a certain extent agrees with my own observations. This statement is also in agreement with the experience of many Fishcurers who tell me that soft fish do not keep so long fresh and are more difficult to cure.

Then there are what is known as "Spawny Haddocks." These have been feeding for some time on Herring spawn, at certain seasons of the year. Now Fishermen and Curers inform me that such fish are very difficult to keep fresh and show early signs of decomposition. Hence it is possible that the kind and quality of the food of fishes so differs in different parts of the sea and at different seasons of the year as to exercise some influence in determining not only the earlier or slower onset of decomposition, but also the quality of the flesh of the fish and its curing and edible properties.

Temperature. As one would expect, temperature plays a very important part in all processes of putrefaction and decomposition in fish. At freezing point, it is supposed, these processes are, if not destroyed, at least inhibited. But I find that fish ungutted and packed in ice, when removed from the iced condition, always begin to exhibit commencing signs of decomposition earlier than fish which have been gutted immediately on capture, and then packed in ice. Hence it is quite possible that the degree of cold produced by icing fish,

as it is generally carried out on board trawlers, may not altogether inhibit fermentative changes taking place in the gut.

From these observations, I conclude that the chief factors concerned in determining the rapidity of post mortem digestion and solution of wall of gut, and the rate of progress of ensuing putrefactive changes in gut and adjacent abdominal walls are:-

(1) The quantity of food in stomach at time of capture.

(2) The quality or kind of food in stomach at time of capture.

(3) Temperature at which fish are kept.

After solution of gut wall the intestinal ferments and Bacteria pass out at once into the peritoneal cavity. But even if the fermentative processes are inactive and solution of gut delayed, the intestinal juices and Bacteria gradually pass out through the dead membrane into the peritoneal cavity, and the ultimate result is the same in both cases. This process is readily followed by cultural and microscopic examination of the peritoneal fluid. Once or twice, in a microscopic preparation of fresh peritoneal fluid, I observed what appeared to be a small rod-shaped bacillus with rounded ends, and also some cocci-like bodies. But on continuing the examination of fresh peritoneal fluid obtained from Haddocks, Whitings, Cod and Skate, by searing the abdomen and withdrawing by sterile platinum wire, immediately after death, I found that when plated out and

incubated on ordinary media, such as Gelatine, Agar, Conradi and Digralski, peritoneal fluid obtained in this way is sterile. However, although the peritoneal fluid is sterile at death, it is remarkable how soon thereafter one will find in it micro-organisms. From 30 to 45 minutes, one will find frequently the Bacillus coli commis, while in one to two hours, these and other Bacteria will be found in considerable numbers.

The functions of the peritoneum are subjects around which there has been much discussion. But on one thing Bacteriologists are now agreed, that during life the peritoneum exercises a strong protective influence against intruding Bacteria. After death the power being lost, the intestinal ferments and Bacteria pass through it very rapidly, and come into close contact with the muscular tissue of the abdominal walls, which usually in a few hours thereafter begin to exhibit a series of changes which are very marked and definite. First, the surface of the muscles and especially those near the neck, which form the "lugs" begin to show a fine pinkish tint which gradually deepens to a reddish brown, and finally to a dark yellowish amber or apple jelly colour. Simultaneously as rigor mortis passes off, the muscles begin to soften, and this softening of the inner surface of the thinner parts of the abdominal walls, combined with the above mentioned discoloration, is spoken of in the fish curing trade, in the case of the Haddock, as Jelly Lugs. If this condition is well advanced, the Fishcurer knows that such fish are not fresh, and often they are difficult to

cure, especially as Findon Haddocks.

If this pulpy,apple jelly like material be examined microscopically, it will be found to consist chiefly of muscle fibres considerably swollen, breaking up into discs, and in process of disintegration. Of the numerous Bacteria always present, the Bacteria Coli is not infrequent, and readily detected in cultures - especially on the Conradi and Digralski Media.

In some cases these processes go on so rapidly that there may be complete digestion of a part of abdominal wall in 36 hours or even less after death. In 48 hours to 72 hours, it may occur in about one half, and in very few will the abdominal wall remain intact after 96 hours.

Undoubtedly, as rigor mortis passes off, this process of auto-digestion in gut and surface of adjacent abdominal walls is early accompanied by, and soon finally superseded by putrefactive processes, the presence of which is readily detected by:-

(1) The softening and apple jelly appearance of abdominal walls.

(2) The increasing stale odour, becoming offensive and finally putrid.

(3) The reaction of the muscles, becoming alkaline to litmus paper.

(4) Sometimes in ungutted fish the presence of Hydrogen Sulphide can be detected.

At this stage Bacteria are always present and can be detected either by microscope or very readily by making cultures from the tissues.

The above statement has reference chiefly to ungutted fish. Here the removal of the gut immediately after capture, or at least soon after capture, will to a considerable extent preclude the process of auto-digestion. Consequently the abdominal muscles remain longer firm, and discoloration with its accompanying softening and putrefaction are delayed. The micro-organisms present in culture from the softening abdominal muscles will also differ from those in ungutted fish, inasmuch as the Bacillus coli will usually be absent.

Ungutted fish during the cold season may keep sufficiently fresh for one or even three days, but if kept longer, whether iced or uniced, the flesh becomes saturated with acrid ferments and exhibit a sour smell. When such fish are cooked, they are found to have lost much of their natural flavour. If cured, it will be found, that, not only have they lost flavour, but also much of their keeping-properties.

THE DISTRIBUTION OF THE BACILLUS COLI IN FISH.

For some time it has been well known that, in man, this Bacillus is the chief inhabitant of the small Intestine and also in the large Intestine it finds a habitat associated with many other micro-organisms. Recently with the development and more extensive application of Sanitary and Bacteriological Science - especially in the consideration of Food and Water Supplies - this Bacillus has been studied more widely in Nature, and has now been proved to be present in the dejecta of most, if not of all mammals; and, that with the exception of some slight differences in culture, in pathogenicity, and as regards fermentation of the different sugars, there is no essential Biological difference between the Bacillus Coli found in man and in the lower mammals.

It has frequently been attempted to formulate these differences as a basis for differentiation and classification of the Bacillus Coli found in the intestine of man and those found in lower animals, and to extend its application to the consideration of Sewage pollution, Water Supplies, etc. But, however desirable this may be, in considering many Public Health questions, in practice it fails; and at the present moment there is no reliable means of distinguishing between Bacillus Coli derived from animal excreta, and those derived from human excreta.

During the past few years, this enquiry has been extended to the lower sub-kingdoms - Birds, Amphibians and Fishes - and

of these, Fishes have probably received the most consideration on account of their extensive and valuable use as a Food stuff, and because they are the inhabitants of waters from which water-supplies are derived.

Houston, in his Report to the Royal Commission on Sewage Disposal, with special reference to the Contamination of Shell Fish, states, as regards Oysters; and in a later Report 1903-04, on the Bacteriological examination of the excreta of Fish - both Oysters and Fish derived from deep sea water remote from sewage pollution - that typical Bacillus Coli or even atypical Bacillus Coli are seldom detectable in the former, and are absent, or only present in small numbers in the Intestines of Fish.

At the same time, the Commissioners had to regret the paucity of our knowledge as to the distribution of the Bacillus Coli in nature.

Recently, Eyre, MacConkey and Johnson have done a considerable amount of work in this direction. Their results differ from those of Houston in this respect, that they find the Bacillus Coli almost universally present in the intestinal canal of Fish.

During the past 9 months, in the intervals of other work, and as opportunity afforded, I have examined the intestinal contents obtained from a large number of different kinds of Fish, with the view of ascertaining the extent of the distribution of the Bacillus Coli in Fish.

The Media used:-

General Media
- (A) MacConkey's Bile-Salt Glucose peptone Litmus solution.
- (B) Drigalski and Conradi's Nutrose Litmus Agar,

and to a less extent

Neutral-red Bile-Salt Agar and Lactose Litmus Agar.

(A) MacConkey's Bile-Salt Glucose peptone litmus solution

Sodium Taurocholate	5 grams.
Glucose	5 grams
Peptone	20 grams
Water	1000 c.c.

The constituents are heated until dissolved, then filtered and sufficient neutral litmus solution added. The Medium is then distributed into Durham's Fermentation tubes, and sterilized by steaming for 20 minutes for three successive days.

(B) Conradi Drigalski Agar Medium.

This medium is rather tedious to prepare.

(1) Agar preparation - To 3lbs. finely minced horse flesh or minced ox beef, add 2 litres of water and let stand 24 hours. Then boil 1 hour and filter. To filtrate, add Peptone Sicca and Nutrose, 20 grams of each, and 10 grams sodium chloride. Boil one hour and filter. Then add 70 grams bar agar, boil for 3 hours in Koch or 1 hour in Autoclave; render slightly alkaline

(using litmus paper), filter, boil for half an hour.

(2) Litmus Solution. Kubel and Tiemann's got from Grübler, Leipsig, is the best. Take 260 c.c., boil for 10 minutes. Then add 30 grams pure milk-sugar and boil 15 minutes.

(3) Take Solution (2) and add to solution (1) when cooled to 60°C. Shake, render faintly alkaline. Then add 4 c.c. hot sterile solution of 10 per cent. water-free soda and 20 c.c. of freshly prepared solution of 0.1 gram Crystal Violet (Hochst) in 100 c.c. warm sterile distilled water.

PARTICULAR MEDIA FOR SUB-CULTURES.

(1) Gelatine slope, stab and shake cultures.

(2) Litmus Milk.

(3) Lactose-peptone Litmus Solution.

(4) Peptone Water.

(5) Glucose Neutral-Red Broth.

In this way, I followed Houston's "Flaginac" basis of classification for Bacillus Coli:-

Fl - Greenish fluorescence in neutral-red cultures.

A.G. - Acid and Gas in Lactose-peptone cultures.

In - Indol formation in peptone cultures.

A.C. - Acid and Clotting of Litmus milk.

A microbe which presents these characters in sub cultural tests is indistinguishable as regards the tests employed, from the typical Bacillus Coli of the human intestine.

Method of procedure.

The fish examined were obtained at different times and from different sources, at the Aberdeen Fish Market during my visitations - some line-caught - some from Trawlers. But the great majority of the fish were not caught at any great distance from the shore, although some were obtained from Trawlers from the Iceland Fishing grounds.

Each fish was washed in tap water, then in sterile water and tacked out on a board. The skin on the ventral aspect of abdomen was thoroughly seared with a red-hot cautery iron, and the stomach and intestines dissected out by sterile instruments. Some of the intestinal contents were then aspirated up into a pasteur pipette or removed by sterile platinum wire to Bile-salt glucose peptone Litmus in a Durham's Fermentation Tube and incubated for 48 hours at 37°C., 1-5 tubes inoculated for each fish.

Sometimes the whole gut was removed, washed in tap water, then in sterile water and the whole contents well mixed with Bile-salt glucose Broth.

From this emulsion other Bile-salt glucose peptone tubes were inoculated with varying quantities. None of the fish were cut up and mixed with the intestinal contents. A positive reaction is shown by the production of Acid and Gas. The acid turns the medium red, and gas is seen in the inner small tube. But as this reaction is only presumptive evidence of the presence of a member of the Coli group, I always plated out on

one or other of the solid media - chiefly Drigalski and Conradi, and from the colonies on this media, when present, sub-cultures were made in accordance with the "Flaginac" basis.

The following Tables exhibit my recorded results.

TABLE I.

SHOWING THE BIOLOGICAL CHARACTERS OF THE BACILLUS COLI AND COLI-LIKE MICROBES ISOLATED FROM FISH.

		Bile-Salt Glucose peptone Litmus Solution 48 Hrs. at 37°C.	Drigalski and Conradi Litmus Agar 24 Hrs at 37°C.	Glucose Neutral red Broth fl - greenish yellow fluorescence 48 Hrs. at 37°C.	Lactose Litmus peptone solution. A.C. - Acid and Gas. 48 Hrs. at 37°C.	Peptone water for Indol. In - Indol. 7 days at 37°C.	Litmus Milk Culture. A.C. - Acid and Clot. 5 days at 37°C.
				Fl.	A.G.	In	A.C.
	4	x	x	x	x	x	x
	2	-	-				
Haddocks	1	-	-				
10 Exp^ts.	1	x	x	Fl sl	x	x	Ac Cl sl
	2	x	x	Fl sl	Ac Gas sl	-	Ac Cl sl
	5	x	x	x	x	x	x
	1	x	x	x	Ac No Gas	-	Ac sl Cl sl
Whitings	1	-					
9 Exp^ts.	2	x	x	-	Ac Gas sl	-	Ac No Cl
	3	x	x	x	x	x	x
	2	x	x	Fl sl	Ac No Gas	-	Ac sl Cl sl
Plaice	1	x	x	Fl	A.G.	-	Ac sl no Cl
7 Exp^ts.	1	x ?	-				

		Bile-Salt Glucose peptone Litmus Solution 48 Hrs.at 37°C.	Drigalski and Conradi Litmus Agar 24 Hrs at 37°C.	Glucose Neutral red Broth fl - greenish yellow fluorescence. 48 Hrs.at 37°C.	Lactose Litmus peptone solution. A.G. - Acid and Gas. 48 Hrs. at 37°C.	Peptone water for Indol. In - Indol. 7 days at 37°C.	Litmus Milk Culture. A.C. - Acid and Clot. 5 days at 37°C.
				Fl.	A.G.	In	A.C.
	4	x	x	x	x	x	x
Dabs	2	-	-				
8 Exp^ts.	1	x	x	-	Ac Gas sl	-	Ac No Cl
	1	-					
	3	x	x	x	x	x	x
Sole	1	x	x	Fl	A.G.	x	Ac No Cl
5 Exp^ts.	1	x	x	Fl	A.G.	-	A.C.
	1	x	x	x	x	x	x
Shrimps	1	x	x	Fl sl	Ac No Gas	-	Ac sl Cl sl
4 Exp^ts.	1	-					
	1	x	x	Fl sl	-	-	Ac sl Cl sl
Dog Fish	1	x	-				
2 Exp^ts.	1	x	x	Fl	Ac sl Gas sl	-	Ac sl No Cl
	1	x	x	Fl sl	A.G.	In sl	Ac
Skate	1	-	-				
3 Exp^ts.	1	-	-				

		Bile-Salt Glucose peptone Litmus Solution 48 Hrs.at 37°C.	Drigalski and Conradi Litmus Agar 24 Hrs.at 37°C	Glucose Neutral red Broth fl - greenish yellow fluorescence. 48 Hrs.at 37°C.	Lactose Litmus peptone solution. A.C. - Acid and Gas 48 Hrs. at 37°C.	Peptone water for Indol. In - Indol. 7 days at 37°C.	Litmus Milk Culture. A.C. - Acid and Clot. 5 days at 37°C.
				Fl.	A.G.	In.	A.C.
Catfish	1	x	x	-	Ac No Gas	-	Ac sl Cl sl
2 Expts.	1	-	-				
	2	x	x	x	x	x	x
Crabs	1	x	x	Fl	Ac sl Gas sl	-	Ac No Cl
4 Expts.	1	x	x	-	Ac sl No Gas	-	-
	2	x	x	Fl sl	A.G.	x	Ac
Cod	1	x	-				
4 Expts.	1	x	x	Fl sl	Ac Gas sl	-	Ac sl Cl sl

T A B L E II.

SUMMARY OF RESULTS.

	No. Experiments.	Typical Bacillus Coli	Atypical Bacillus Coli	Negative results.
Haddocks	10	4	3	3
Whitings	9	5	3	1
Plaice	7	3	3	1
Dabs	8	4	1	3
Sole	5	3	2	
Shrimps	4	1	2	1
Dog-Fish	2		1	1
Skate	3	1		2
Cat-Fish	2		1	1
Crabs	4	2	2	
Cod	4	2	1	1
Total	58	25	17	16
Percentage		43%	33%	24%

BACTERIOLOGICAL EXAMINATION of FRESH PERITONEAL FLUID - chiefly for Bacillus Coli.

Method Adopted.

The fish were killed immediately after capture, the ventral aspect of abdomen washed, with sea water in the case of some dealt with at sea; with tap water and sterile water in the case of those caught near shore by line - boat or rod. Then the abdomen was carefully seared with red hot cautery, and peritoneal cavity opened by sterile knife and three loopfulls of peritoneal fluid withdrawn by means of sterile platinum wire and used for the inoculation of each tube of the following media:-

(A) Bile-salt glucose peptone litmus solution in Durham's fermentation tubes.

(B) Drigalski and Conradi's medium directly by stroke method.

25 Experiments were made with the following Fish:-
6 Haddocks, 5 Whitings, 2 Young Skates, 2 Codlings, 2 Cat-Fish, 1 Dog-Fish, 3 Plaice and 4 Herrings.

In 19 cases, the results were negative.

In the case of 2 Herrings, 1 Whiting and 1 Plaice, there appeared in the Bile-salt Medium a very slight trace of discoloration, and just a few bubbles of gas. On the Conradi and Drigalski Medium, one of these Herrings and the Whiting gave negative results. From the remaining Herring and Plaice subcultures were made on various media, but only in the case of the

Herring were any growths obtained, and from these a Streptococcus was isolated. It was noted, however, at time of capture that this Herring did not appear to be in a healthy condition. There were several scratches on its sides and patches of scales had been rubbed off. Two of the cases suffered liability to contamination, and are not here considered.

Although from these few experiments one is neither warranted nor justified in coming to the conclusion that the Peritoneal Fluid in Fish is a sperile fluid, yet, from the fact that out of 23 experiments (leaving out two spoiled experiments), only four suggested some suspicion, and from only one of which an ill-conditioned fish was a Streptococcus isolated, the evidence of these experiments points to the probability of the peritoneal fluid in healthy living fish as being sterile.

I made a series of observations regarding the time after death, when the Bacillus Coli could be detected in the peritoneal fluid.

About 30 experiments were made at different periods. The fish were killed, laid out on trays and kept moist. At short intervals the abdomen was opened with aseptic precautions and suitable media inoculated, incubated, examined and sub-cultures made.

In about 5 cases, the Bacillus Coli was detected 40 minutes after death. In about 9 cases, 1 hour after death, and from 1½ to 2 hours after death, micro-organisms are numerous in the peritoneal fluid.

In consideration of the above results, and those of other workers, it is difficult to avoid the conclusion, that the Coli Bacillus, if not a normal inhabitant of the intestinal canal of Fish in general - a statement which we readily admit is not yet fully warranted from the above observations - has at least a far wider distribution in Fish than was generally anticipated.

Although very little work has been done on this subject in this country, the results of Houston and Eyre are interesting and invite comparison.

Houston, experimenting on Fish obtained off the Norfolk Coast - a locality remote from sewage pollution - found that only 13% of the cases he examined exhibited typical Bacillus Coli; 52% Atypical; while 34% gave negative results. From these results he concludes that, although fish in a sewage-free locality may sometimes contain typical Bacillus Coli in their interior, it can hardly be the case that even Coli-like microbes are present naturally in abundance in Fish.

Eyre*, working about the same time, and finding the Coli Bacillus almost universally present in the intestinal canal of the lower mammals and birds, obtained a large variety of Fish off the Lincolnshire coast, and had no difficulty in isolating typical Bacillus Coli from every fish experimented with.

It is thus quite reasonable to suppose that either a sewage-polluted or a sewage-free environment will exercise some influence in determining the extent to which Bacillus Coli or Coli-like organisms may be present in Fish inhabiting such

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* Lancet, March 1904.

localities. I have examined several varieties of Fish caught in Aberdeen Bay, round the area where sewers discharge, and in many of these the colonies of the Coli Bacillus were present in enormous numbers in the Media used; whereas in the case of Fish known to be caught some distance from land, the colonies were generally few in number.

Houston, working on Guillemots and Gulls, found that whereas the intestinal contents of the former, in culture, yielded negative results as regards the Bacillus Coli, Bacillus Enteritidis Sporogenes and Streptococci, the intestinal contents of the latter contained Bacillus Coli in enormous numbers, and also although not so numerous, Bacillus Enteritidis Sporogenes and Streptococci - the intestinal organisms closely associated with the Coli Bacillus. He ascribes this result to the different habits of feeding; Guillemots are clean feeders, whereas Gulls feed on all sorts of filth.

In this respect, the study of the life history of the Mussel is very interesting and instructive. For its varying life conditions appear to have a considerable influence in determining its wholesomeness or unwholesomeness.

Some investigations were made by Virchow and Schmidtmann and by Wolff and Konig. The former found that when poisonous mussels were left in pure sea-water they became harmless in less than one month. The latter showed that if non-poisonous Mussels were placed near a sewage outlet, or even in the water of a Harbour, they became poisonous in about two weeks. If now these Mussels are transferred into the neighbourhood of a

sluice, where the water is frequently changed, they very soon again become harmless.

These considerations raise some very important questions. It is possible that the Bacillus Coli may not be a natural nor a necessary inhabitant of the intestinal canal in certain lower animals, but may have become so, by finding a temporary lodgment through the exigencies of living and feeding, and then by laying aside the saprophytic and taking on the parasitic habit; while as already shown, the number of coli present in a fish depends largely on topographical considerations influencing the type of feeding.

Such questions as these, and the now recognised possibility of Birds, Fish, etc., as important Bacillus Coli carriers, infecting one another, polluting oyster beds, rivers and streams, foodstuffs and water-supplies; as also the different views held, whether all kinds of excremental pollution is potentially dangerous to health, or whether it is only dangerous in degree, as it is dejecta from a human source, and finally the very great influence which the consideration of such matters is destined to exercise on many Public Health questions, e.g. - is the presence of Bacillus Coli synonymous with sewage pollution - I will not discuss here, as it is not within the scope of this paper. But I may add, in conclusion, that, since for the present the Bacillus Coli is accepted as the best indicator of excremental pollution, while at the same time Bacteriology fails to distinguish between Coli Bacillus from human sources and those derived from the lower animals, and since from the Public

Health point of view, the one or the other has quite a different significance; the Bacteriologist in future, having due regard to all local conditions, will require to interpret his results more in a relative and less in an absolute sense. For it is only by a judicious weighing of the Bacteriological results with all the topographical data that an approximately reasonable and correct estimate can be formed of the potential danger to health, regarding many of the important questions which have so frequently to be considered by Public Health Authorities.

SUMMARY REGARDING DETECTION OF DECOMPOSITION IN FISH.

After discussing the various factors concerned in the decomposition of fish and the various criteria ordinarily applied in its detection, the important question still remains, how far these criteria are applicable and reliable in the hands of those whose daily duties are the examination of fish. Although it may be conceded at once that it is probably unsafe to consider any one of these criteria as individually absolutely reliable, yet in reviewing the whole question, I am inclined to consider the following five tests as fairly reliable in giving comparatively trustworthy evidence as regards the condition of a fish:-

I. PRESENCE OR ABSENCE OF RIGOR MORTIS.

II. PRESENCE, DEGREE OF DEVELOPMENT OF, OR ABSENCE, OF REDDISH DISCOLORATION ON VENTRAL ASPECT OF BACKBONE.

III. SMELL.

IV. MANNER IN WHICH FLESH SEPARATES FROM THE BACKBONE.

V. APPEARANCE OF ABDOMINAL WALLS.

I. So long as a fish is in the condition of Rigor Mortis, it is a guarantee that it is perfectly fresh, since decomposition can only set in as rigor passes off: the ordinary tests for which already enumerated - Degree of Rigidity on handling and balancing; flesh firm and elastic and does not pit readily on pressure. The chemical changes in the muscle are also important - Acid during Rigor, becoming alkaline as Rigor passes

off and finally distinctly alkaline when decomposition has set in - both to litmus paper. But since under the most favourable conditions under which fish are treated, rigor mortis is of short duration, its absence is no guarantee that fish are not sufficiently fresh and not fit for human food.

II. At this stage the presence or absence of reddish discoloration on ventral aspect is invaluable and should always be looked for. If it is present, we know that the fish are certainly not quite fresh. The time will probably be about 48 to 60 hours after capture or after landing. But even at this stage the fish may not be such as should be condemned as unfit for human food or for curing purposes. Yet, when one sees this discoloration fully developed, it should make one suspicious and more cautious as regards the condition and examine them more critically by further tests. Also, it has to be kept in mind that to prevent this discoloration, an attempt is sometimes made to remove the large caudal vein along with the gut.

III. The sense of smell in the examination of fish is invaluable in spite of the difficulties already discussed. I have attempted to describe smell in terms of Fresh, Fishy and Seaweedy for one large class of fish; as Fresh, Fishy and Oily in another large class of fish, and to contrast these with such terms in every day use as tainted, stale and putrid. Although one at the same time recognises the different and relative degrees of development of the sense of smell, and consequently

the difficulty in getting unanimity in different individuals of what constitutes these different terms, yet the test of smell is both a time-honoured and reliable standard. One will usually find that, as the red discoloration is appearing, the smell is passing from fresh to tainted and stale. The fish is now on the border-land, and one smells critically for an approaching putrid odour, when the fish should be at once condemned.

IV. When a fish is fresh, it requires considerable pressure to strip the flesh from the backbone, and in doing so many tags of flesh are left adhering to the bone. As decomposition and consequently softening progresses, the flesh gradually strips off cleaner. Hence, when one finds that the flesh comes away readily and comparatively cleanly from the bone, or that the bone can be stripped readily and cleanly from the flesh, one may feel convinced that the fish are certainly not fresh; that decomposition, if not well advanced, has certainly commenced; and by this and other tests proposed, one will feel warranted in condemning such fish.

V. In examining interior of abdominal cavity, one notes condition of kidney situated anteriorly and ventral to backbone. It is a very diffuse, vascular and friable organ, and very rapidly breaks down - passing through different shades of colour - to form a reddish-brown debris in 24-48 hours, while the fish may be still quite fresh. But more important is the

condition of abdominal walls. If they are firm and elastic with absence of discoloration, and presence of fresh, characteristic smell, one may feel assured that the fish are fresh. On the other hand, if the walls are soft and pulpy, with apple-jelly-like appearance, and presence of discoloration with tainted odour, while the flesh is becoming alkaline to litmus paper, then such fish require very careful consideration, and it will generally be found, that, with other confirmatory evidence present, such fish should be condemned.

Other common tests which should never be omitted are:-

VI. APPEARANCE OF THE GILLS.

The gills of most fish are red in colour with certain specific tints. These tints disappear in about 24 to 36 hours, and the gills become grey and slimy by the 3rd to 4th day. So long as the gills retain their natural colour, there is a strong presumption that the fish are fresh. But one has to keep in view that the gills often retain their characteristic colour with little change - especially if washed daily in tap or better sea-water - even when the flesh is becoming putrid; that on the whole the gills of trawled fish are often paler at time of capture than line fish and more so the longer they have been in the trawl net; also, that one finds degrees of paleness even amongst perfectly fresh fish.

VII. APPEARANCE OF THE EYE.

The appearance of the eye should always be noted. The full and prominent eye with jet black pupil and transparent cornea of the fresh fish presents a very decided contrast with the grey and shrunken eye of a fish four or five days after capture.

VIII. APPEARANCE OF SCALES.

One notes the absence or presence of characteristic sheen; the firmness or looseness of the scales, and if they rub off readily. If the scales present a patchy appearance, it indicates that the fish are probably trawled or have been roughly handled.

IX. GENERAL APPEARANCE.

In looking at a fish, the appearance it presents often indicates whether it is a trawled or line fish. In the former the body region generally shows a battered and limp appearance, with often considerable extravasation of blood in the head region.

From the above considerations, I venture to state that when:-

(1) Rigor Mortis has passed off,

(2) Reddish discoloration fully developed as described, and seen in Diagram,

(3) Smell becoming tainted - passing to putrid,

(4) Flesh strips off readily and cleanly from backbone,

(5) Abdominal walls becoming soft and pulpy with commencing apple jelly-like appearance and with commencing discoloration and tainted odour,

(6) Gills lost characteristic tint - becoming gray and slimy,

(7) Eyes gray and shrunken,

such fish should unhesitatingly be condemned.

I have purposely refrained from appending a Bibliography of modern Literature on this subject. Because on the one hand one would require to include a vast literature with very little direct bearing on the special subjects here discussed; or, on the other hand, if one is to include only such literature as deals directly with the subject of fish, it would be very small.

But I have found of great value:-

1. The Yearly Reports of The Fishery Board for Scotland.
2. The Yearly Reports of the Local Government Board,- chiefly the Bacteriological Articles on many different subjects by Houston.
3. Several Articles by MacConkey on the Differentiation and Isolation of the Bac: Coli communis.

Amongst writers on this subject I ought to mention -

Cossar Ewart - The Preservation of Fish.

Johnson - Isolation of Bac: Coli from Intestinal Tract of Fishes - Journal of Infectious Diseases, Vol.1. p. 348.

Eyre - Distribution of Bac: Coli in Nature, Lancet, 1904.

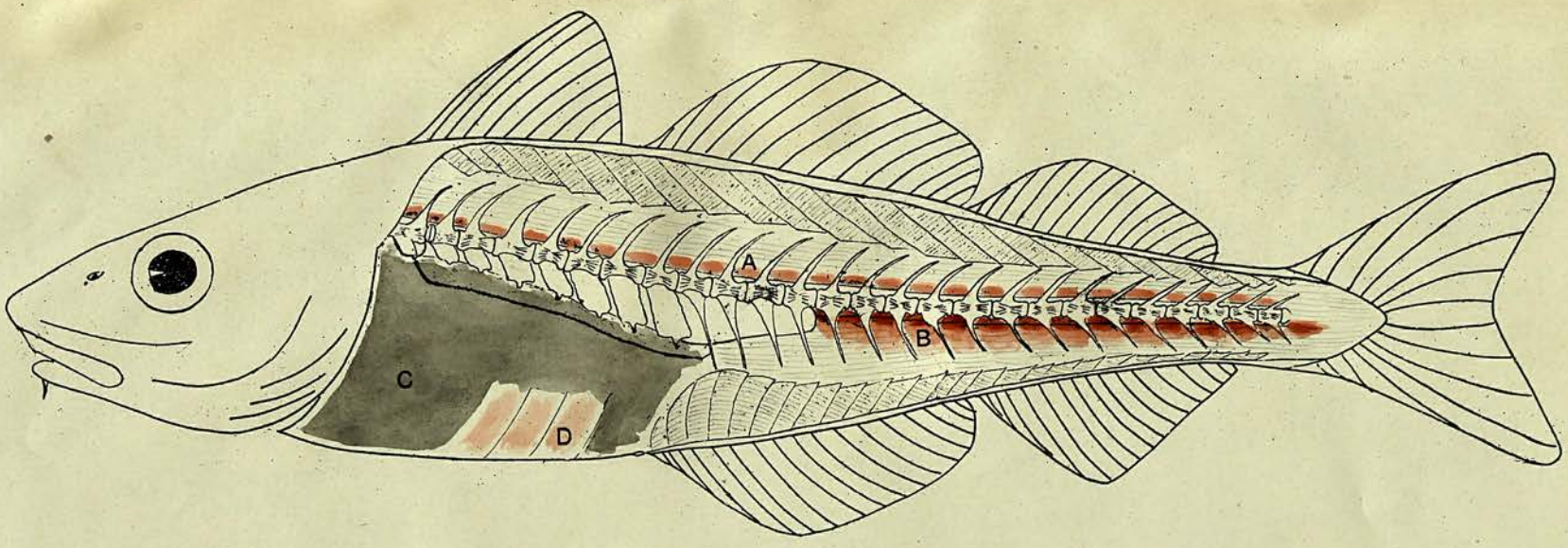

DIAGRAM OF HADDOCK

About Three Days after capture

(A) Slight reddish discoloration due to Dorsal Aorta.

(B) Deeper reddish discoloration due to Caudal Vein on ventral and posterior aspect of Abdominal Cavity.

(C) Peritoneum.

(D) Peritoneum removed and Abdominal Walls breaking down, showing pale apple jelly colour—"Jelly Lugs."